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# (54) PROTEIN AMSH AND CDNA THEREOF

(57) This application provides a human protein AMSH having the amino acid sequence of SE ID No. 1 which is a novel signal transduction molecule interacting with the SH3 domain of cytokine based signal transduction molecule STAM; a gene encoding the above AMSH; a cDNA having the nucleotide sequence of SEQ ID No. 2; and antibody against AMSH.

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## Description

### Technical Field

[0001] The present invention relates to human protein hAMSH and mouse protein mAMSH, and cDNAs encoding these proteins. More particularly, the present invention relates to novel human and mouse signal transduction molecules, AMSH, human and mouse genes encoding these proteins, cDNAs thereof, and antibodies against these proteins.

### Background Art

[0002] A variety of cells having different functions should collaborate with each other for expression of higher biological functions such as hematopoietic, immunological and nervous systems. Communication among the cells is essential for their collaborations. Cytokines are known to be the proteins responsible for intercellular communication, and include interleukin (IL)-1 to 18, colony stimulation factors (CSFs), interferons (IFNs) and several chemokines.

[0003] Signals are generated by binding of the cytokines to specific receptors on the cell membrane, and survival, proliferation, differentiation and functional expression of the cells are controlled by signal transduction. Accordingly, dysfunction of cytokine-receptor signal transduction pathways result in collapse of the immunological and hematopoietic systems to cause severe infectious diseases, cancers and autoimmune diseases.

[0004] For the reasons described above, it is quite important to elucidate the intracellular signal transduction pathways mediated by the cytokine/cytokine receptor system. This is important, in order to appreciate the basic phenomena such as proliferation and differentiation of the cells, pathogenesis, diagnosis and therapeutic intervention of various diseases at the molecular levels.

[0005] The inventors of the present invention have isolated the genes of "common γ-chain" commonly included in a plurality of the cytokine receptors, and have made a great contribution in elucidating the structure and function of the cytokine receptors. Of particular elucidation is that the γ-chain is an essential subunit for functional expression of IL-2, IL-4, IL-7 and IL-9, and abnormality in the γ-chain causes impairment of early development of T-cells via dysfunction of IL-7 (Science, 262:1874-1877, 1993; Int. Immunol., 6:1451-1454, 1994; Science, 263:1453-1454, 1994; Eur. J. Immunol., 25:3001-3005, 1995).

[0006] Recently, the inventors of the present invention have identified "STAMs" as novel signal molecules concerning signal transduction in the proliferating cells by the cytokines, and found that these STAMs are present downstream of the IL-2/GM-CSF receptor and directly associate with JAK3/2. The inventors also found that STAMs play an important role in expression of c-myc and signal transduction for DNA synthesis (Immunity, 6:449-457, 1997).

[0007] While several important mechanisms of the intracellular signal transduction pathway by binding of the cytokines to the receptors have been made clear, additional novel molecules should be identified for elucidating the overall structure and function of the intracellular signal transduction pathway, because plural molecules are thought to be continuously and synergetically involved in the signal transduction pathway and to manifest final functional expression by constructing a so-called cascade.

[0008] The present invention was performed by taking the above situations into consideration, and the object of this invention is to provide a novel signal transduction molecules interacting with SH3 domain of a signal molecule STAM that has been found by the present inventors, and exerting essential functions on signal transduction to the downstream from STAM.

[0009] Another object of the present invention is to provide gene encoding this novel molecule, cDNA thereof, and antibody against the novel molecule.

### Disclosure of Invention

[0010] The present invention for solving the foregoing problems provides a human protein hAMSH having the amino acid sequence of SEQ ID No. 1

[0011] The present invention also provides a human gene encoding the human protein hAMSH, hAMSH cDNA having the nucleotide sequence of SEQ ID No. 2, and a DNA fragment comprising a partial sequence of SEQ ID No. 2.

[0012] The present invention further provides a recombinant vector containing the cDNA or the partial fragment thereof, and an antibody against the human protein hAMSH.

[0013] The present invention additionally provides a mouse protein mAMSH having the amino acid sequence of SEQ ID No. 3, a mouse gene encoding the mouse protein mAMSH, mAMSH cDNA having the nucleotide sequence of SEQ ID No. 4, a DNA fragment comprising a partial sequence of SEQ ID No. 4, a recombinant vector containing the cDNA or the DNA fragment thereof, and an antibody against the mouse protein mAMSH.

**Best Mode for Carrying Out the Invention**

**[0014]** The procedure for obtaining a human protein hAMSH and cDNA thereof, as well as the procedure for identifying the function of the protein will be described hereinafter.

**[0015]** The human protein hAMSH cDNA according to the present invention is a human gene's cDNA isolated by screening a human cDNA library by Far-Western method using a chimera gene of SH3 domain of STAM gene and glutathione-S-transferase (GST). This cDNA has a nucleotide sequence comprising 1910 base pairs shown in SEQ ID No. 2, and encodes the protein hAMSH having the amino acid sequence of SEQ ID No. 1.

**[0016]** While estimated a nucleus transfer signal and a JAB1-like structure are observed in the molecule of the protein hAMSH, this protein is confirmed to be a novel molecule since corresponding molecules are not found in the protein data base.

**[0017]** The facts that this protein hAMSH is a novel molecule involved in signal transduction for cell proliferation by association with STAM, downstream of the cytokine receptor, is confirmed as follows:

(1) AMSH-dc2 in which half of the C-terminal region of hAMSH has been deleted, suppresses signal transduction for DNA synthesis after stimulating with IL-2 and GM-CSF; and
(2) The AMSH-dc2 mutant suppresses c-myc inducing signal transduction after stimulating with IL-2 and GM-CSF.

**[0018]** The cDNA of the mouse protein mAMSH according to the present invention is a mouse gene's cDNA isolated by screening a mouse cDNA library using the hAMSH cDNA as a probe. This cDNA has the nucleotide sequence comprising 1384 base pairs shown in SEQ ID No. 4, and encodes the protein mAMSH having the amino acid sequence of SEQ ID No. 3.

**[0019]** The proteins hAMSH and mAMSH according to the present invention may be obtained by conventional methods such as isolating from human or mouse organs or from cell lines, preparing the peptides by a chemical synthesis based on the amino acid sequence provided by the present invention, or by a recombinant DNA techniques using the cDNA fragments provided by the present invention. For example, in order to obtain the protein hAMSH by the recombinant DNA technique, RNA is prepared by in vitro transcription from the vectors comprising cDNA fragment having the nucleotide sequence of SEQ ID No. 2, and the protein may be expressed through in vitro translation using the RNA as a template. The protein encoded by the cDNA may be also expressed in large scale in E. coli, Bacillus subtilis, yeast, animal cells and plant cells by recombining an expression vector with the translation region of cDNA by known methods in the art.

**[0020]** When the protein according to the present invention is produced by expression of DNA by in vitro translation, the cDNA or its translation region is recombined into a vector having a RNA polymerase promoter, and the recombinant cDNA is added in an in vitro translation system such as a rabbit dyalyzate or wheat germ extract containing a RNA polymerase for the promoter. Examples of the RNA polymerase promoter include T6, T3 and SP6. Examples of the vector containing these RNA polymerase promoter include pKA1, pCDM8, pT3/7 18, pT7/3 19 and pBlueprint II.

**[0021]** For large scale production of the protein encoded in cDNA in microorganisms such as E. coli, an expression vector is constructed by inserting the cDNA according to the present invention or its translation region into an expression vector comprising an origin suitable for microorganisms, a promoter, a ribosome binding site, cDNA cloning sites and terminater, followed by cultivation of the transformant cell obtained after transformation of the host cell with the expression vector. A protein containing an arbitrary region can be obtained by allowing the expression vector to express by adding an initiation codon and a stop codon before and after the arbitrary translation region. Otherwise, a desired protein portion can be selectively obtained by allowing the protein to express as a fusion protein with other protein, followed by cleaving this fusion protein with an appropriate protease. Examples of the expression vector for use in E. coli include pUC, pBluescript II, pET expression system and pGEX expression system.

**[0022]** For producing the protein according to the present invention in eukaryotic cell such as animal cells, the cDNA or the translation region thereof is inserted into an expression vector for the eukaryotic cell comprising a promoter, splicing region and poly (A) addition site to introduce the recombinant vector into the eukaryotic cell. Examples of the expression vector include pKA1, pCDM8, pSVK3, pMSG, pSVL, pBK-CMV, pBK-RSV, EBV vector, pRS and pYES2. While cultured cells of a mammal such as monkey kidney cells COS7 and Chinese hamster ovary cells CHO, budding yeast, dividing yeast, silkworm cells and African clawed frog egg cells are usually used as the eukaryotic cells, any eukaryotic cells may be used so long as they are able to express MIST. The expression vector can be introduced into the eukaryotic cell by a conventional method such as an electroporation method, a calcium phosphate method, a liposome method and a DEAE dextran method.

**[0023]** Separation procedures known in the art may be combined for isolating and purifying the desired protein from the culture after expressing the protein in microorganisms or eukaryotic cells. The separation and purifying methods include, for example, treatment with a denaturation reagent such as urea or with a surface active agent, ultrasonic treatment, enzymatic digestion, salting-out and solvent precipitation method, dialysis, centrifugation, ultrafiltration, gel

filtration, SDS-PAGE, isoelectric point electrophoresis, ion exchange chromatography, hydrophobic chromatography, affinity chromatography and reversed phase chromatography.

**[0024]** Peptide fragments (five amino acid residues or more) containing any partial amino acid sequence of the amino acid sequence of SEQ ID Nos 1 and 3 are included in the proteins hAMSH and mAMSH according to the present invention. These peptide fragments may be used for producing antibodies. Fusion proteins with other proteins are also included in the protein according to the present invention.

**[0025]** The gene of the present invention is a human gene encoding the protein described above, and can be isolated, for example, from the existing genome library using the cDNA of the present invention or a partial sequence thereof as a probe.

**[0026]** The cDNA of the present invention may be obtained by screening a cDNA library derived from a human cell or mouse cell through colony or plaque hybridization known in the art by using the oligonucleotide probe synthesized, based on the nucleotide sequence of SEQ ID Nos. 2 and 4. Alternatively, the cDNA of the present invention can be prepared from the mRNA isolated from a human cell or mouse cell by the RT-PCR method using synthesized oligonucleotides that can hybridize to both ends of the cDNA fragment as primers.

**[0027]** Polymorphism due to individual differences is often observed in the animal gene. Accordingly, cDNAs containing an addition or deletion of one or plural nucleotides, and/or substitution with other nucleotides in SEQ ID Nos. 2 and 4 are also included within the scope of the present invention.

**[0028]** Likewise, the proteins containing an addition or deletion of one or plural amino acids, and/or substitution with other amino acids caused by the alteration of cDNAs may be also included within the scope of the present invention, provided that the protein comprises protein activity pf the protein having the amino acid sequence of SEQ ID No. 1 or 3.

**[0029]** The DNA fragments (10 bp or more) comprising any partial nucleotide sequence of SEQ ID No. 2 or 4 are also included in the DNA fragment of the present invention. DNA fragments comprising sense strand and antisense strand may be categorized into the DNA fragment of the present invention. The DNA fragments can be used as a probe for gene diagnosis.

**[0030]** Antibodies against the proteins of the present invention may be obtained as polyclonal antibodies or monoclonal antibodies by conventional methods using the proteins themselves or partial peptides thereof as antigens.

## Example

**[0031]** A part of the hAMSH cDNA (the 383-550th in SEQ ID No. 2: corresponds to the amino acids 125-180th in SEQ ID No. 1) was amplified by PCR and was inserted into the GST fusion protein expression vector. This vector was introduced into E coli for transformation, and this transformant was stimulated with IPTG to induce expression of the GST fusion protein. The induced fusion protein was purified by affinity chromatography using a glutathione column, thereby obtaining the GST fusion protein. An antiserum was obtained by immunizing a rabbit with this GST fusion protein as an antigen.

## Industrial Applicability

**[0032]** As hitherto described in detail, the present invention provides novel signal transduction molecules related to the cytokine based signal transduction pathway, and gene engineering materials. These molecules and gene engineering materials are useful for developing diagnostic and therapeutic methods as well as pharmaceuticals for human diseases due to dysfunction of the cytokine based signal transduction pathway such as severe infectious diseases, cancers and autoimmune diseases.

SEQUENCE LISTING

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275 280 285

Leu Met Arg Asn Glu Phe Thr Ile Thr His Val Leu Ile Pro Lys Gln

290 295 300

Ser Ala Gly Ser Asp Tyr Cys Asn Thr Glu Asn Glu Glu Glu Leu Phe

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Leu Ile Gln Asp Gln Gln Gly Leu Ile Thr Leu Gly Trp Ile His Thr

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acca 1384

## Claims

1. A human protein hAMSH having the amino acid sequence of SEQ ID No. 1.
2. A human gene encoding the human protein hAMSH of claim 1.
3. cDNA of the human gene of claim 2, which is hAMSH cDNA having the nucleotide sequence of SEQ ID no. 2.
4. A DNA fragment comprising a partial sequence in the nucleotide sequence of SEQ ID No. 2.
5. A recombinant vector containing the hAMSH cDNA of claim 4 or the DNA fragment of claim 4.
6. An antibody against the human protein hAMSH of claim 1.
7. A mouse protein mAMSH having the amino acid sequence of SEQ ID No. 3.
8. A mouse gene encoding the mouse protein mAMSH of claim 7.
9. cDNA of the mouse gene of claim 7, which is mAMSH cDNA having the nucleotide sequence of SEQ ID no. 4.
10. A DNA fragment comprising a partial sequence in the nucleotide sequence of SEQ ID No. 4.
11. A recombinant vector containing the mAMSH cDNA of claim 9 or the DNA fragment of claim 10.
12. An antibody against the mouse protein mAMSH of claim 7.

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP99/06309

A. CLASSIFICATION OF SUBJECT MATTER
Int.Cl[7] C07K 14/47, C12N 15/12, C07K 16/18, C12P 21/08 //
C12N 1/21, C12P 21/02, (C12N 15/12, C12R 1:91),
(C12P 21/02, C12R 1:19)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
Int.Cl[7] C12N 15/00-90, C07K 14/00-16/46, C12P 21/00-08, C12N 1/00-38

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
MEDLINE (STN), Genbank/EMBL/DDBJ/GeneSeq, WPI (DIALOG), BIOSIS (DIALOG)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| PX<br>PY | Kazuo Sugamura et al., "Possible involvement of a novel STAM-associated molecule "AMSH" in intracellular signal transduction mediated by cytokines", J.Biol.Chem. (July 1999), Vol.274, No.27, p.19129-19135 | 1- 6<br>7-12 |
| X | Wei Yu et al., "Large-scale concatenation cDNA sequencing", Genome Research (1997), Vol.7, No.4, p.353-358 | 4,5,10,11 |
| X | Meredith A.Wentland et al., "A"Double Adaptor"method for improved shotgun library construction", Analytical Biochemistry (1996), Vol.236, No.1, p.107-113 | 4,5,10,11 |
| A | Sugamura K.et al., "STAM, signal transducing adaptor molecule, is associated with Janus kinases and involved in signaling for cell growth and c-myc induction", Immunity(1997), Vol.6, No.4, p.449-457 | 1-12 |

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
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